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Species Concepts in Pteridophytes: The Treatment and Definition of Agamosporous Species

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Pteridophyte species have generally been defined on the basis of relatively major morphological differences between sets of populations. At least implicitly, these morphological discontinuities are taken to indicate lack of gene flow and are thought to reflect the genetic discontinuities that make one species distinct from another. When functionally diploid, outcrossing fern species are circumscribed in this way, morphologically recognizable taxonomic species may also be good biological species. Unfortunately, in the case of agamosporous ferns (formerly designated "apogamous" or "apomictic"), the criteria used to define a biological species do not apply. Unlike the members of a biological species, agamosporous individuals cannot interbreed. However, they can cross with related sexually reproducing taxa to generate reproductively competent offspring, which biological species are not supposed to do. Thus the reproductive behavior of agamosporous ferns precludes application of a strict biological species concept, and the treatment and definition of agamosporous species is somewhat problematical. This issue merits consideration because agamosporous taxa constitute about 10% of all fern species for which the type of reproduction is known (Walker, 1984 p. 125). In this paper, we review the salient features of the typical life cycle of agamosporous pteridophytes. We then discuss the origins of several agamosporous fern taxa and indicate how we would treat them taxonomically. We conclude with a species concept accommodating both sexual and agamosporous taxa.

MATERIALS AND METHODS

Electrophoretic samples of *Notholaena grayi* Davenp. and the *Pellaea atropurpurea* (L.) Link complex were obtained from living plants maintained in greenhouses at the University of Kansas. Enzymes were extracted by crushing a small section (ca. 50 mm^2) of immature leaf tissue in ten drops of the phosphate grinding buffer-PVP solution of Soltis et al. (1983). The grindate was absorbed into paper wicks which were inserted into 12.5% starch gels for electrophoresis. Phosphoglucomutase (PGM) was resolved on gel/electrode buffer system 6 of Soltis et al. (1983). Leucine aminopeptidase (LAP), hexokinase (HK), and triosphosphate isomerase (TPI) were resolved on the modification of buffer system 8 discussed by Haufler (1985). Malate dehydrogenase (MDH) was resolved using a modification of gel/electrode buffer system 11 (Soltis et al., 1983) in which the concentration of histidine-HCl was doubled. Shikimate

dehydrogenase (SkDH) and isocitrate dehydrogenase (IDH) were resolved using the 0.04M citrate buffer of Clayton & Tretiak (1972) titrated to a pH of 7.5 with N-3(3-aminopropyl)-morpholine. TPI, HK, SkDH, and IDH were assayed using the agarose staining schedules of Soltis et al. (1983). PGM, LAP, and MDH were assayed using recipes provided by Werth (1985). Stained gels were photographed using a red filter and Kodak Technical Pan 2415 high contrast film.

Results and Discussion

Background.—To understand why agamosporous taxa cannot interbreed and yet can cross with related sexually reproducing taxa to produce new, true-breeding agamosporous lineages, one must be conversant with details of the agamosporous life cycle. These details have been well documented (Manton, 1950) and recently reviewed (Lovis, 1977; Walker, 1979, 1984). The life cycle of obligately agamosporous ferns involves alternation of two quite separate phenomena—the avoidance of meiotic reduction in sporogenesis (producing diplospores and gametophytes at the same ploidy level as the sporophyte that begets them) and the spontaneous development of a new sporophyte from the gametophyte without fertilization (apogamy or apomixis). We follow Löve & Löve (1975) in adopting the term "agamospory" for this overall reproductive process involving both diplospory and apogamy.

At least two different major variations in the sporogenetic part of the agamosporous life cycle are known (Lovis, 1977; Walker, 1979). Because the implications for speciation and the recognition of species in agamosporous ferns are the same no matter which sporogenetic system operates, we will concentrate on the more commonly encountered Döpp-Manton scheme since the examples from our work follow this pattern. Sporogenesis in this system (Fig. 1) begins with the archesporial cell undergoing three successive mitotic cell divisions so that the sporangium contains eight cells of sporophytic ploidy. Thereafter two alternative courses are followed. In one type of sporangium (Fig. 1, upper sequence) the eight cells undergo a fourth mitotic division, yielding sixteen spore mother cells with the sporophytic chromosome complement. During meiosis, the chromosomes in these cells fail to pair regularly, instead forming univalents, bivalents, and multivalents. As a result, the chromosomes are not distributed evenly, and the resultant spores are chromosomally unbalanced and abortive. In the other type of sporangium (Fig. 1, lower sequence), the fourth mitotic division of the eight cells starts normally. The chromosomes gather on the equator and divide, but there is no nuclear or cell division. Instead, a restitution nucleus is formed having double the original chromosome number. Thus at the end of the fourth division (Fig. 1, heavy arrow) there are eight spore mother cells, each with twice the sporophytic ploidy. Because the fourth division was endomitotic, each chromosome now has an identical sister chromosome with which to pair. Pairing is therefore regular as the eight spore mother cells undergo meiosis, and sporogenesis yields 32 viable spores with the same chromosome number as the sporophyte.

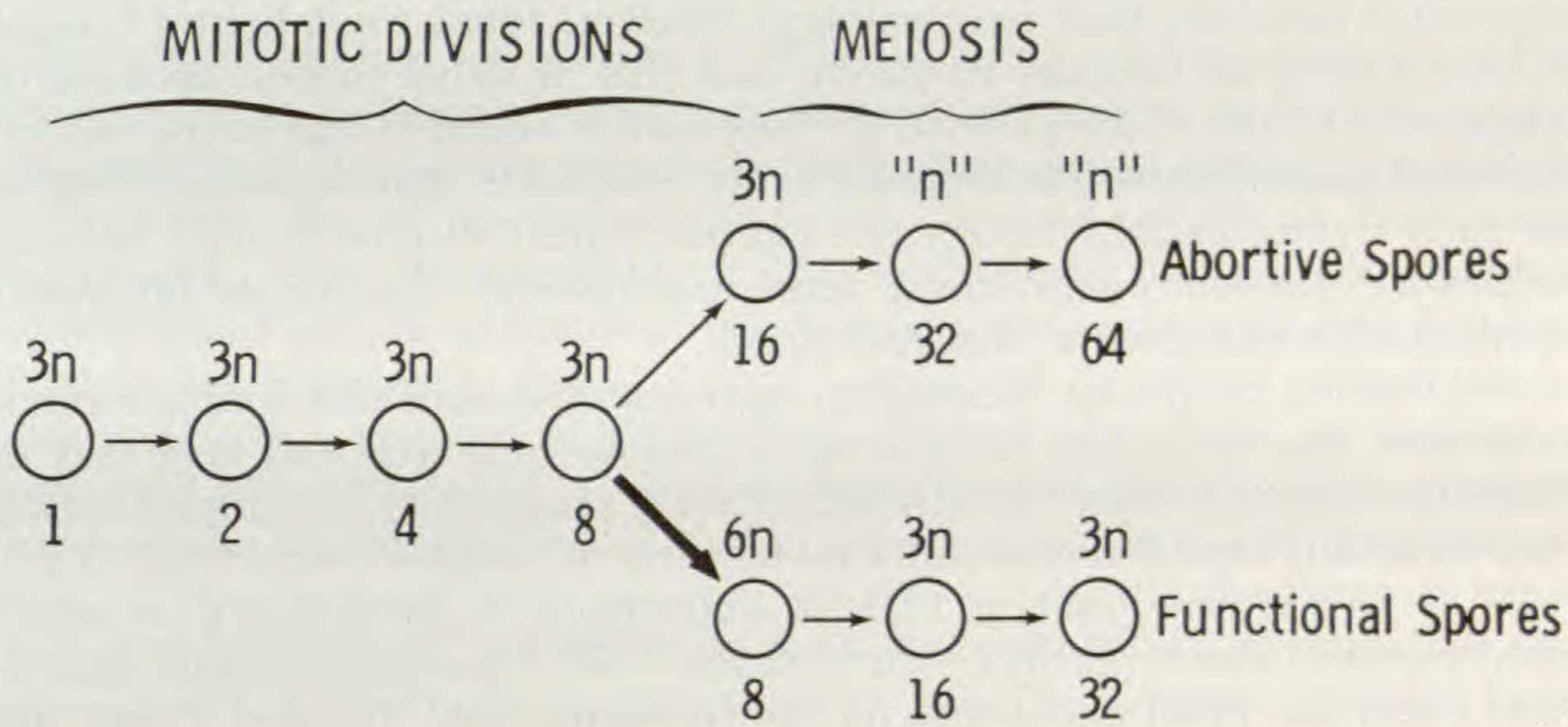


FIG. 1. Diagrammatic representation of sporogenesis in the Döpp-Manton agamosporous system. Circles represent cellular stages. Arrows represent the divisions of mitosis and meiosis. 3n denotes the sporophytic chromosome complement at whatever ploidy. Numbers below circles indicate the number of cells at that stage in the process. Heavy arrow represents the irregular mitotic division (endomitosis) leading to a restitution nucleus. See text for further explanation.

A few agamosporous taxa such as *Notholaena grayi* and *N. aliena* show variations in the Döpp-Manton scheme that result in the formation of 16 spores per sporangium (Windham, unpubl. data), and some sexually reproducing ferns commonly produce 32-spored sporangia (Vida et al., 1970; Hickok & Klekowski, 1974; Smith, 1974). Therefore, spore counts may be used to generate hypotheses concerning the life cycle of a given taxon, but the existence of agamospory must be verified chromosomally or by growing gametophytes and observing sporophyte production in the absence of fertilization.

When restitution nuclei of momentarily doubled ploidy undergo meiosis in Döpp-Manton sporogenesis (Fig. 1), the pairing of sister chromosomes precludes genetically significant recombination and segregation. Barring mutation, the genotype of each spore is identical to that of the parental sporophyte. The unreduced spores are disseminated and develop into gametophytes that look normal except that (1) functional archegonia are suppressed and (2) a new sporophyte develops spontaneously from gametophytic tissue without fertilization. The absence of syngamy means that no genetic variation is introduced through the union of genetically dissimilar gametes. These factors make it reasonable to assume that once an agamosporous lineage is established, it is essentially clonal, genetically invariant except for non-deleterious mutations.

Although lack of functional archegonia and syngamy precludes the introduction of variant genetic material into an agamosporous lineage, Döpp-Manton agamosporous taxa can produce antheridia with functional (non-reduced) sperm and can thereby act as male parents in crosses with archegoniate gametophytes of sexually reproducing taxa. Hybrids resulting from such crosses inherit the complete agamosporous mechanism and are therefore able to

reproduce faithfully their new genotype (Walker, 1966). Such hybrid lineages always feature an increase in ploidy over that of either parent, because the unreduced ploidy of the paternal gametophyte is added to that of the reduced maternal gametophyte. The reproductively competent, agamosporous offspring of such hybridizations bridge the morphological discontinuities between otherwise discrete evolutionary lines and thereby complicate taxonomic identification and species circumscription.

Application of species concepts to agamosporous taxa and their taxonomic treatment depend in part on how agamosporous taxa arise and how they are therefore related to their sexually reproducing progenitors. In the past, writers have generally regarded agamosporous fern taxa as being of hybrid origin (Lovis, 1977, p. 389; Walker, 1984, p. 127). This conclusion is based partly on meiotic chromosome behavior in the 16-spore-mother-celled sporangia of agamosporous taxa (Manton, 1950) and partly on the reasoning that "there is a very high proportion of triploid cytotypes which can only have arisen by hybridization" (Walker, 1979, p. 117). The assertion that triploid cytotypes can only have arisen by hybridization overlooks the possibility that triploid or other polyploid cytotypes might be autopolyploids that have arisen through intraspecific fertilization in which one or both gametophytes are diploid because they are derived from unreduced spores.

To determine whether polyploid cytotypes do arise in nature via gametophytes from unreduced spores, Gastony (1986) tested Morzenti's (1967) complex hypothesis that tetraploid *Asplenium plenum* results from a cross between an unreduced triploid gametophyte of *A. curtissii* and a haploid gametophyte of *A. abscissum*. The mechanism of unreduced spores was tested in an allopolyploid system rather than an autopolyploid one because variant species-specific electrophoretic markers could be identified and traced in an allopolyploid phylogeny whereas such markers would necessarily not be available in an autopolyploid phylogeny. Gastony's electrophoretic data matched expectations under Morzenti's hypothesis and rejected the competing hypothesis, confirming that unreduced spores in nature do produce gametophytes that generate polyploid sporophytic cytotypes. Unreduced gametophytes have also been implicated in the origin of scattered triploid sporophytes of *Cystopteris protrusa* (Haufler et al., 1985), *Woodsia mexicana* and *Bommeria hispida* (Windham & Haufler, 1985). Thus, it is clear that unreduced spores do yield sexually functional unreduced gametophytes in nature that are capable of crossing with haploid or other diploid gametophytes to produce triploid or tetraploid sporophytes. In the absence of other evidence of interspecific hybridization, the triploid and tetraploid cytotypes of agamosporous taxa need not be explained by interspecific hybridization. Instead, they may be autopolyploids (intraspecific polyploids) derived through the mechanism of naturally occurring unreduced spores.

If at least some agamosporous fern taxa are autopolyploids, why do the endomitotic spore mother cells of their 32-spored sporangia show normal bivalent formation rather than multivalents? In an agamosporous autotriploid, for example, these cells would contain six homologous chromosome sets and

multivalents should be observed. This situation could be explained if chromosome associations during meiosis were dependent on both structural homology and the action of certain regulatory genes. Genetic control of chromosome pairing has been reported for several species of *Asplenium* (Braithwaite, 1964; Bouharmont, 1972a, 1972b), and preliminary evidence suggests that it may be widespread among agamosporous taxa exhibiting Döpp-Manton sporogenesis. Rigby (1973) identified trivalent chromosome associations in sporangia of *Pellaea atropurpurea* that failed to undergo a premeiotic endomitosis (Fig. 1, upper sequence), suggesting that the three sets of chromosomes in this agamosporous triploid are at least partially homologous. However, endomitiotic sporangia (Fig. 1, lower sequence) from the same plant yielded only bivalents, providing no indication that all six chromosome sets were capable of associating to form multivalents. The same situation has been observed in several other agamosporous triploids (Windham, unpubl. data), including *Asplenium monanthes*, *Cheilanthes bonariensis*, *Cheiloplecton rigidum*, *Notholaena aschenborniana*, and *Argyrochosma limitanea*. Although the genetic mechanism is poorly understood, it appears that multivalent formation in these ferns is suppressed whenever an even number of homologous genomes is present in the cell. If such control of pairing is common among agamosporous taxa (as preliminary evidence suggests), the absence of multivalents during meiosis cannot be used as evidence against autopolyploid origins of agamosporous ferns.

Case studies.—Mode of origin, degree of genetic continuity with sexual progenitors, reproductive interactions, and morphological distinctions must all be taken into consideration when characterizing agamosporous taxa and determining their taxonomic treatment. The following examples from our work on the relationships of agamosporous taxa in *Pellaea*, *Notholaena*, and *Cheilanthes* illustrate the value of modern biosystematic data when attempting to generate meaningful species concepts for this problematical group of pteridophytes.

Pellaea andromedifolia, endemic to California and Baja California Norte, includes widespread sexually reproducing diploid populations and sympatrically interspersed, agamosporously reproducing triploid and tetraploid populations (Tryon, 1957, 1968; Gastony & Gottlieb, 1985). Sporophytes from sexual and agamosporous populations of all ploidy levels look alike. The only way to distinguish them morphologically is by counting spores per sporangium, with 64 in the sexuals and 32 in agamosporous individuals. Thus there is no morphological evidence of interspecific hybridization in the origins of the polyploids.

Details of electrophoretically detected genetic variation in natural populations of *P. andromedifolia* of both reproductive types were provided by Gastony & Gottlieb (1985). Comparative electrophoresis showed that agamosporous triploid sporophytes and their respective gametophyte progenies are genetically identical. This confirmed the lack of recombination and segregation expected from chromosome pairing behavior in Döpp-Manton sporogenesis. Furthermore and most importantly, all alleles coding allozymes in

agamosporous populations were entirely a subset of those in the sexual populations. Thus there is no electrophoretic evidence that hybridization with another species was involved in their origin. As in the *Asplenium plenum* complex, reproductive interactions involving gametophytes from unreduced spores of *P. andromedifolia* could account for both their triploidy and their possession of only *P. andromedifolia* electrophoretic bands.

A similar situation was observed during investigations of *Notholaena grayi* (Windham, unpubl. data), a species found in the southwestern U.S. and adjacent Mexico. Tryon (1956) indicated that *N. grayi* produced 32 spores per sporangium, but a survey of herbarium specimens identified some populations having only 16 spores per sporangium. Subsequent cytogenetic work revealed that 32-spored plants were sexual diploids whereas those with 16 spores per sporangium were agamosporous triploids. As in *Pellaea andromedifolia*, the two cytotypes are morphologically very similar, suggesting that interspecific hybridization was not a factor in the origin of the agamosporous taxon. Preliminary electrophoretic data for 19 enzyme loci support this conclusion. Figures 2–7 illustrate zymogram patterns of *Notholaena grayi* for the enzymes PGM, LAP, TPI, SkDH, IDH, and MDH. In each of these figures, the two lanes at the far left and the two on the far right represent sexual diploids, and the intervening lanes show all of the variation so far encountered among agamosporous triploids. For the loci coding PGM-2, LAP, TPI, SkDH, IDH, MDH-1, and MDH-2, all alleles found in the agamosporous individuals were also detected among the sexual diploids. The only orphan allele (found in the triploid but not in the diploid) occurred at the locus coding the cathodal MDH isozyme (MDH-3) in two plants from a single population (Fig. 7). This allele may be found upon further sampling of the diploid, or it may represent a recent mutation in the triploid that has not yet spread beyond the population of origin. In either case, morphological and electrophoretic data suggest that the agamosporous triploid form of *Notholaena grayi* arose through autopolyploidy.

Further insight into the relationships between agamosporous taxa and their sexual progenitors is provided by the *Pellaea glabella* complex. At the time of the last taxonomic revision of this group (Tryon, 1957; Tryon & Britton, 1958), it was said to consist of three varieties: (1) sexual diploid var. *occidentalis* in South Dakota, Wyoming, Montana, and Alberta, (2) agamosporous tetraploid var. *simplex* in Alberta, British Columbia, Washington, Utah, Colorado, Arizona, and New Mexico, and (3) agamosporous tetraploid var. *glabella* widely distributed in eastern North America. Tryon (1957) suggested either allopolyploid or autopolyploid origins for the agamosporous varieties. The allopolyploid hypothesis proposed that sperm of agamosporous triploid *P. atropurpurea* fertilized a haploid egg of centrally distributed sexual *P. glabella* var. *occidentalis*, yielding agamosporous tetraploid var. *glabella* to the east and agamosporous tetraploid var. *simplex* to the west. Tryon's autopolyploid hypothesis derived both agamosporous varieties directly from var. *occidentalis*, the only sexual diploid known to her.

Several years later, Wagner et al. (1965) discovered a sexual diploid race of *Pellaea glabella* var. *glabella* in Missouri that was indistinguishable from

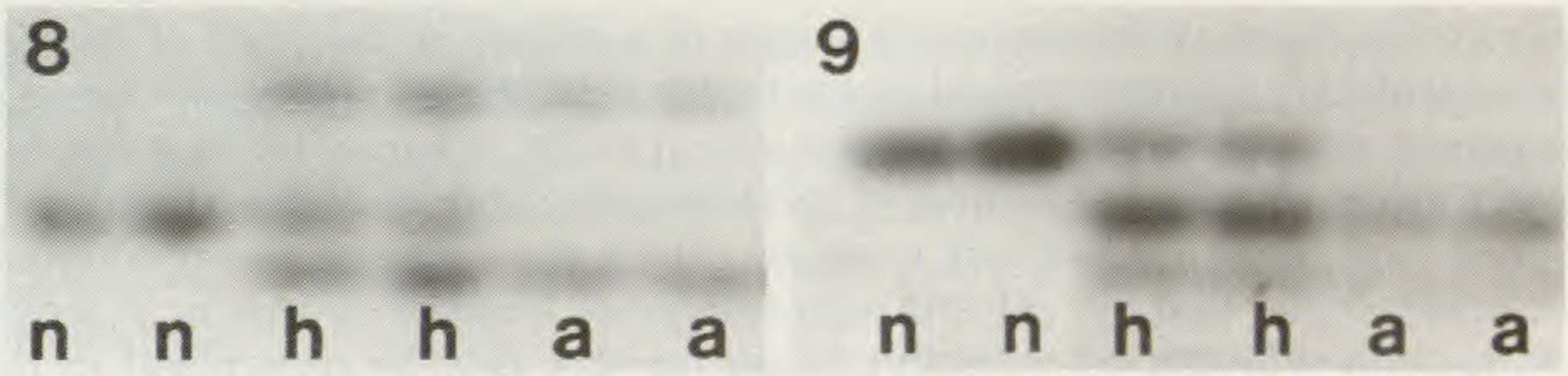
FIGS. 2–7. Zymograms of *Notholaena grayi*. Numbers in the margin identify different isozymes in enzymes coded by multiple loci. In each figure, the two lanes on the far left and the two on the far right represent sexual diploids. Intervening lanes represent agamosporous triploids.

agamosporous tetraploid var. *glabella* except in its chromosome number and number of spores per sporangium. They suggested that this new sexual race made an allopolyploid origin for agamosporous var. *glabella* unlikely, proposing instead that the agamosporous tetraploid was an autotetraploid derivative of the sexual diploid of the same variety.

Comparative enzyme electrophoretic data for *P. atropurpurea* and the sexual and agamosporous taxa in the *P. glabella* complex were presented by Gastony (1988). All sampled populations of agamosporous var. *simplex* were invariant for the analysed enzyme patterns. Variation in allozyme patterns was found among the populations of agamosporous var. *glabella*, and the chromosome numbers of all such populations confirmed that they represent truly tetraploid agamosporous variety *glabella* and not variant backcrosses to sexual plants. These electrophoretically variant tetraploid populations may represent primary apomicts that have accumulated mutations since their origin or populations that have arisen through independent origins from sexual individuals with different genetic constitutions. Electrophoretic data further showed that *P. atropurpurea* was not involved in the origin of either agamosporous tetraploid.

Based on population samples of the four *glabella* taxa from throughout their ranges, Gastony (1988) found that, like agamosporous varieties *simplex* and *glabella*, the two sexual taxa have several distinctive allozyme patterns for the enzymes considered. He concluded that (1) the enzyme banding patterns of agamosporous tetraploid var. *simplex* are contributed by sexual var. *occidentalis* and that (2) the somewhat variable banding patterns of agamosporous tetraploid var. *glabella* are a subset of those in sexual var. *glabella*. As in the case of *P. andromedifolia* and *Notholaena grayi*, there are no distinctive enzyme bands that have been contributed by another species and thus no electrophoretic evidence to support a hypothesis that these agamosporous tetraploids arose through interspecific hybridization. In fact the electrophoretic data are consistent with the hypothesis that they are autotetraploids derived from the respective sexual diploids presumably through the mechanism of unreduced spores.

Although *Pellaea atropurpurea* was not involved in the origin of either agamosporous tetraploid variety of *P. glabella*, ongoing studies indicate that much of the morphological variation observed in *P. atropurpurea* results from hybridization between these triploid plants and sympatric sexual taxa. Electrophoretic and chromosomal data reveal that the so-called "simple form" of *P. atropurpurea* (discussed and illustrated by Tryon, 1972) arose through hybridization between that species and the rare Mexican diploid, *P. notabilis*. The resultant agamosporous tetraploid is fully fertile and has spread throughout much of Mexico (Windham, unpubl. data). It is morphologically intermediate between the putative parents and shows additivity at 10 enzyme loci, including SkDH (Fig. 8) and HK (Fig. 9). *Pellaea atropurpurea* has also hybridized with *P. truncata* in the southwestern U.S., producing an agamosporous tetraploid with more dissected leaves and sparsely pubescent rachises. At one locality in Oklahoma, *P. atropurpurea* has even hybridized with the sexual tetraploid *P. wrightiana* to form several pentaploid plants of intermediate morphology.



FIGS. 8–9. Zymograms documenting the hybrid origin of the "simple form" of *Pellaea atropurpurea*. A = *P. atropurpurea*; N = *P. notabilis*; H. = hybrid. 8. SkDH. 9. HK.

Similar evidence of hybridization between *P. atropurpurea* and *P. glabella* var. *occidentalis* has been observed in South Dakota, Wyoming, and Alberta (Gastony, 1988). *Pellaea atropurpurea* is by no means unique in its tendency to hybridize with sympatric congeners. Hybridization between agamosporous species and sexual relatives has also been reported in *Pteris* (Walker, 1962), *Phegopteris* (Mulligan et al., 1972), and *Asplenium* (Morzenti, 1966).

An additional example of hybridization revolves around the agamosporous tetraploid "form" of *Cheilanthes wootonii* provisionally called *C. yavapensis* by Reeves (1979). *Cheilanthes wootonii* is an agamosporous triploid (Windham, 1983), and one would assume that it was involved in the origin of the tetraploid "form" of that species. However, electrophoretic data (Reeves & Windham, in prep.) suggest that *C. "yavapensis"* is a hybrid between *C. lindheimeri* (an agamosporous triploid quite distinct from *C. wootonii*) and *C. covillei* (a sexual diploid). This illustrates well the taxonomic confusion that can result when electrophoretic data are lacking for reproductively competent, agamosporous-sexual hybrids.

Application to species concepts.—Several conclusions relevant to the treatment and definition of agamosporous species emerge from the foregoing case studies. (1) Once an agamosporous taxon is initiated, its genotype is perpetuated and not disrupted by meiotic segregation, recombination, or syngamy. (2) Individuals or populations that are electrophoretically detectable genetic variants of the rest of an agamosporous taxon at its base ploidy may reflect mutations that have accumulated in these populations since a single origin event or they may indicate multiple origins of the taxon from genetically variant sexual progenitors. (3) At least some agamosporous taxa at the primary level (such as those in *P. andromedifolia, Notholaena grayi* and the *P. glabella* complex) appear to be of autopolyploid origin. Taxonomic treatment of agamosporous lineages must recognize these genetic realities if taxonomy is to reflect phylogeny. How we would treat agamosporous lineages taxonomically is exemplified by the following discussion of the taxa used in our case studies.

Some autopolyploid primary agamosporous lineages are genetically indistinguishable from their sexual progenitors at the enzyme electrophoretic level (except for banding pattern fixation caused by non-segregation and gene dosage effects associated with polyploidy) and are morphologically indistinguishable (except for number of spores per sporangium). Genetic

diversity in these agamosporous lineages is a subset of the pool of genetic diversity of the progenitor sexual taxon. In this case, the agamosporous taxon is not genetically discontinuous from the sexual taxon in a qualitative sense but merely quantitatively so by virtue of its extra gene set or sets. The situation in *Pellaea andromedifolia* is an example. Here genetic and morphological continuity argue that sexual and agamosporous lineages represent a single species with different reproductive behaviors that may be recognized in the sporophytes only by counting spores, counting chromosomes, or observing electrophoretic banding patterns. The case of the sexual and agamosporous lineages of *P. glabella* var. *glabella* is analogous to that in *P. andromedifolia*. Here also electrophoretically detectable genotypes of the agamosporous tetraploid comprise a subset of the allelic variation in the sexual diploid, and except for counting spores or chromosomes the two lineages cannot be distinguished reliably (Gastony, 1988). As in *P. andromedifolia*, the distinction between these taxa seems best recognized at the level of varieties. Comparable taxonomic treatment may be appropriate for the autotriploid agamosporous lineage of *Notholaena grayi*.

Other autopolyploid agamosporous lineages may show similar qualitative genetic continuity with their sexual progenitors (being fixed for a subset of the sexuals' alleles, although with extra doses of them) but show slight divergence from the sexuals in other respects. In the case of *P. glabella* var. *simplex* versus var. *occidentalis*, there is sufficient morphological discontinuity (even if partially attributable to a gigas effect in the tetraploid) that the sexual and agamosporous lineages have long been recognized as distinct varieties or even species (Brunton, 1979; Lellinger, 1985). In this case, qualitative genetic continuity again argues against distinction at the species level, but a slight morphological discontinuity (with an underlying, although undetermined, genetic basis) usually permits distinguishing the two taxa without counting spores or chromosomes. In addition, these lineages are almost completely allopatric. The fact that var. *simplex* survives fairly well under greenhouse cultural conditions in Indiana whereas var. *occidentalis* suffers great mortality under identical cultural conditions suggests that these taxa also differ physiologically. This degree of divergence of the sexual and agamosporous taxa is greater than that in *P. andromedifolia* and has accordingly been recognized at slightly higher rank, viz. at the level of subspecies (Gastony, 1988).

In the case of agamosporous triploid *P. atropurpurea*, distinctive enzyme electrophoretic patterns indicate genetic discontinuity with other *Pellaea* taxa, and this is paralleled by its morphological discontinuity. We do not yet have enough data to determine whether it is of autopolyploid or allopolyploid origin. Although its agamosporous lifestyle prevents interbreeding among plants of its own lineage, electrophoresis does reveal genetic variation throughout its range, even at the triploid level. It therefore features the genetic characteristics of a sexual "biological" species (coherent genetic variation that is discontinuous with the coherent genetic variation of other species) and correlated morphological distinctness from other species. It can hybridize with sexual taxa and thereby produce reproductively functional hybrids because of momentary

chromosome doubling just before meiosis in Döpp-Manton sporogenesis. In this it hardly differs from "good," sexually reproducing, "biological" plant species that are able to hybridize and whose hybrid offspring become reproductively functional if their chromosome number doubles. Agamosporous taxa such as *P. atropurpurea* are appropriately recognized at the rank of species.

Agamosporous lineages can and do act as male parents in crosses with sexually reproducing relatives. The offspring of such crosses are agamosporous at a ploidy level higher than that of their agamosporous parent and are capable of reproducing and perpetuating their new lineage through agamospory. Tetraploid *Cheilanthes "yavapensis"* with a substantial geographic range from southern New Mexico to northern Arizona provides an example of such an allopolyploid origin. Its geographic range and reproductive capability indicate a degree of evolutionary performance typical of successful species, and we consider it appropriate to recognize taxa such as this at the rank of species. The respective hybrids of *Pellaea atropurpurea* with *P. notabilis* and with *P. truncata* also belong to this category.

If the derivative of an agamosporous-sexual cross consists only of one or a few individuals resulting from isolated, independent hybridization events, it may be treated taxonomically simply as an occasional hybrid plant, a potential lineage that has not yet demonstrated evolutionary performance warranting acceptance as a species. Individuals of this kind can be accommodated under the hybrid formula name uniting the epithets of their parents with a "×." This, for example, may be the preferred treatment for the agamosporous derivative *Pellaea atropurpurea* × *wrightiana*.

We regard a species as a coherent evolutionary lineage whose allele frequencies change through time as its genome varies in response to selection and other perturbations. This is a genetic species concept in which the species is genetically equivalent to that in the widely accepted biological species concept. In the biological species concept, two mechanisms maintain the genetic integrity of the species: (1) panmixis or potential panmixis maintains the genetic coherence of the species, and (2) the inability to produce fertile offspring in reproductive interactions with other species under natural conditions maintains genetic discontinuity with other species. For sexually reproducing taxa, the occasional production of hybrids does no violence to this concept when the hybrids are sterile. When chromosome doubling renders these hybrids fertile and they perpetuate their lineage, often with range expansion as in some members of the Appalachian *Asplenium* complex (Wagner, 1954), we have no difficulty in accepting them as species of hybrid origin, while still regarding the parental taxa as species.

In our genetic species concept, agamosporous species are genetically equivalent to sexual species in the biological species concept, but the mechanisms by which they maintain their genetic integrity are different. The absence of both syngamy and genetically significant recombination replace panmixis in maintaining the genetic coherence of the species, and the inability to produce fertile hybrid offspring at the same ploidy level in paternal interactions with sexual species maintains genetic discontinuity with other

species. Thus the genetic species concept is equally applicable to both sexual and agamosporous taxa and its genetic consequences are comparable to those of the biological species concept. This genetic approach to species definition permits us to formulate treatments of agamosporous taxa of various derivations. These treatments can be both genetically meaningful and taxonomically practical. Species of *Pellaea*, *Notholaena*, and *Cheilanthes* provide examples in which agamosporous taxa are appropriately recognized as species, subspecies, and varieties when data from morphology, cytogenetics, and enzyme electrophoresis are given combined consideration.

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Literature Cited

Bouharmont, J. 1972a. Meiosis and fertility in apogamously produced diploid plants of *Asplenium trichomanes*. Chromosomes Today 3:253–258.

———. 1972b. Meiosis in apogamously produced diploid plants of *Asplenium septentrionale*. Brit. Fern Gaz. 10:237–240.

Braithewaite, A. F. 1964. A new type of apogamy in ferns. New Phytol. 63:293–305.

Brunton, D. F. 1979. Taxonomy, distribution, and ecology of the cliff-brake ferns (*Pellaea*: Polypodiaceae) in Alberta. Canad. Field-Naturalist 9:288–295.

Clayton, J. W. and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Canada 29:1169–1172.

Gastony, G. J. 1986. Electrophoretic evidence for the origin of fern species by unreduced spores. Amer. J. Bot. 73:1563–1569.

———. 1988. The *Pellaea glabella* complex: electrophoretic evidence for the derivations of the agamosporous taxa and a revised taxonomy. Amer. Fern J. 78:44–67.

——— and L. D. Gottlieb. 1985. Genetic variation in the homosporous fern *Pellaea andromedifolia*. Amer. J. Bot. 72:257–267.

Haufler, C. H. 1985. Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. 10:92–104.

———, M. D. Windham, D. M. Britton, and S. J. Robinson. 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. Canad. J. Bot. 63:1855–1863.

Hickok, L. G. and E. J. Klekowski. 1974. Inchoate speciation in *Ceratopteris*: an analysis of the synthesized hybrid *C. richardii* × *C. pteridoides*. Evolution 28:439–446.

Lellinger, D. B. 1985. *A field manual of the ferns & fern allies of the United States & Canada*. Washington: Smithsonian Institution Press.

Löve, A. and D. Löve. 1975. *Plant Chromosomes*. Vaduz: J. Cramer.

Lovis, J. D. 1977. Evolutionary patterns and processes in ferns. Pp. 229–415 in *Advances in botanical research*, Vol. 4, eds. R. D. Preston and H. W. Woolhouse. New York: Academic Press.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: University Press.

MORZENTI, V. M. 1966. Morphological and cytological data on southeastern United States species of the *Asplenium heterochroum-resiliens* complex. Amer. Fern J. 56:167–177.

———. 1967. *Asplenium plenum*: a fern which suggests an unusual method of species formation. Amer. J. Bot. 54:1061–1068.

MULLIGAN, G. A., L. CINQ-MARS, and W. J. CODY. 1972. Natural interspecific hybridization between sexual and apogamous species of the beech fern genus *Phegopteris* Fée. Canad. J. Bot. 50:1295–1300.

REEVES, T. 1979. A monograph of the fern genus *Cheilanthes* subgenus *Physapteris* (Adiantaceae). Ph.D. dissertation, Arizona State University, Tempe, Arizona.

RIGBY, S. J. 1973. Chromosome pairing in obligately apogamous ferns: *Pellaea atropurpurea* and *Pellaea glabella* var. *glabella*. Rhodora 75:122–131.

SMITH, A. R. 1974. Taxonomic and cytological notes on ferns from California and Arizona. Madroño 22:376–378.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9–27.

TRYON, A. F. 1957. A revision of the fern genus *Pellaea* section *Pellaea*. Ann. Missouri Bot. Gard. 44:125–193.

———. 1968. Comparisons of sexual and apogamous races in the fern genus *Pellaea*. Rhodora 70:1–24.

———. 1972. Spores, chromosomes and relations to the fern *Pellaea atropurpurea*. Rhodora 74:220–241.

——— and D. M. BRITTON. 1958. Cytotaxonomic studies on the fern genus *Pellaea*. Evolution 12:137–145.

TRYON, R. M. 1956. A revision of the American species of *Notholaena*. Contr. Gray Herb. 179:1–106.

VIDA, G., C. N. PAGE, T. G. WALKER, and T. REICHSTEIN. 1970. Cytologie der Farn-Gattung *Cheilanthes* in Europa und auf den Canarischen Inseln. Bauhinia 4:223–253.

WAGNER, W. H., JR. 1954. Reticulate evolution in the Appalachian Aspleniums. Evolution 8:103–118.

———, D. R. FARRAR, and K. L. CHEN. 1965. A new sexual form of *Pellaea glabella* from Missouri. Amer. Fern J. 55:171–178.

WALKER, T. G. 1962. Cytology and evolution in the fern genus *Pteris* L. Evolution 16:27–43.

———. 1966. Apomixis and vegetative reproduction in ferns. Pp. 152–161 in *Reproductive biology and taxonomy of vascular plants*, ed. J. G. Hawkes. Bot. Soc. Brit. Isles Conf. Rep. 9.

———. 1979. The cytogenetics of ferns. Pp. 87–132 in *The experimental biology of ferns*, ed. A. F. Dyer. New York: Academic Press.

———. 1984. Chromosomes and evolution in pteridophytes. Pp. 103–141 in *Chromosomes in evolution of eukaryotic groups*, Vol. 2, eds. A. K. Sharma and A. Sharma. Boca Raton, Florida: CRC Press.

WERTH, C. R. 1985. Implementing an isozyme laboratory at a field station. Virginia J. Science 36:53–76.

WINDHAM, M. D. 1983. The ferns of Elden Mountain, Arizona. Amer. Fern J. 73:85–93.

——— and C. H. HAUFLER. 1985. Autopolyploid evolution among homosporous ferns. Amer. J. Bot. 72:919.